

Exhibit 2



Molecular Probes

Handbook of Fluorescent Probes and Research Chemicals

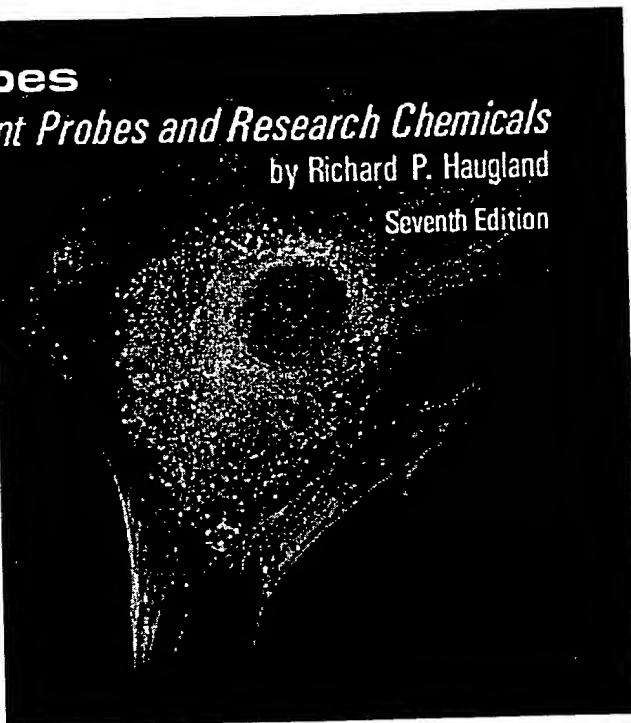
by Richard P. Haugland

Seventh Edition

Table of Contents

Help

Copyright © 1999
by Molecular Probes, Inc.



Handbook of Fluorescent Probes and Research Chemicals

Quick Find



Search

Chapter < Section **Section** Chapter | Products | Data Table |

Table of Contents

A Letter from the PresidentIntroduction to Fluorescence TechniquesChapter 1 — Fluorophores and Their Amine-Reactive DerivativesChapter 2 — Thiol-Reactive ProbesChapter 3 — Reagents for Modifying Groups Other Than Thiols or AminesChapter 4 — Biotins and HaptensChapter 5 — Crosslinking and Photoreactive ReagentsChapter 6 — Fluorescence Detection Methods Including FluoSpheres and ELF TechnologiesChapter 7 — Protein Conjugates for Biological DetectionChapter 8 — Nucleic Acid DetectionChapter 9 — Peptide and Protein Detection, Analysis and SynthesisChapter 10 — Enzymes and Enzyme SubstratesChapter 11 — Probes for Actin, Tubulin and Nucleotide-Binding ProteinsChapter 12 — Probes for OrganellesChapter 13 — Probes for Lipids and MembranesChapter 14 — Fluorescent Tracers of Cell Morphology and Fluid FlowChapter 15 — Assays for Cell Viability, Proliferation and FunctionChapter 16 — Probes for Endocytosis, Receptors and Ion ChannelsChapter 17 — Photoactivatable (Caged) ProbesChapter 18 — Probes for Signal TransductionChapter 19 — Probes for Reactive Oxygen Species, Including Nitric OxideChapter 20 — Indicators for Ca(2+), Mg(2+), Zn(2+) and Other MetalsChapter 21 — pH IndicatorsChapter 22 — Indicators for Na(+), K(+), Cl(-) and Other AnionsChapter 23 — Probes for Membrane PotentialChapter 24 — Fluorescence Measurement Accessories and Resources

1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16
17	18	19	20
21	22	23	24

Technical Notes and Product Highlights

- FluoReporter Kits for Labeling Proteins with a Fluorescent Dye or Biotin
- Fluorescence Resonance Energy Transfer
- Thiol-Reactive Probes Discussed in Other Chapters of the Handbook
- Quantitation of Biotin and Avidin
- Custom Immunogen Preparation
- Limitations of Low Molecular Weight Dyes
- Fluorescent Microspheres for Blood Flow Determination
- Add Free Biotin to Obtain Brighter Signals from Fluorescent Avidin Conjugates
- Staining Nucleic Acids on Plastic Wrap or Paraffin Sheets
- Fluorescent Probes for Photoconversion of Diaminobenzidine
- Lipid Mixing Assays of Membrane Fusion
- Antibodies for Detecting Membrane Surface Labels
- Anti-Lucifer Yellow and Anti-Cascade Blue Antibodies
- Dichroic Mirrors for Simultaneous Photoactivation of Caged Compounds and Visualization of Fluorescent Probes in a Fluorescence Microscope
- Loading and Calibration of Intracellular Ion Indicators
- Assays of Volume Change, Membrane Fusion and Membrane Permeability
- A Sampling of Sampler Kits
- Easy-to-Use Protein Labeling Kits
- MAXC: A Computer Program for Calculating Free Ca^{2+} Concentrations
- The Alexa Fluor Dye Series - Peak Performance Across the Visible Spectrum

 **Top**



Copyright © 1999 by Molecular Probes, Inc. Legal Notices and Trademark Attributions.

A Letter from the President

Dear Researcher:

The Seventh Edition of Molecular Probes' *Handbook of Fluorescent Probes and Research Chemicals* completely updates and expands the Sixth Edition of the *Handbook*, which was published in November of 1996. This CD-ROM edition duplicates much of the information that is available at our popular Web site, including the full text of the *Handbook*, a bibliography with over 32,000 citations linked to our products, chemical structures, full spectra for many compounds, a gallery of over 200 images, product information sheets for many products and much more. Molecular Probes has developed several particularly important new products since the publication of the Sixth Edition of the *Handbook*, including our Alexa Fluor dyes, SYBR Gold nucleic acid stain and our ultrasensitive SYPRO Ruby protein stains — these are fully described in this edition. The text has been completely revised and, in some cases, chapters or sections have been reorganized or combined to make it easier for you to locate product information. In addition, the CD-ROM is completely searchable.

The medium of the CD-ROM is ideal for communicating the wealth of information in the databases that we maintain on our products and their applications. Because the content of this CD-ROM is fixed until we publish another edition, we invite you to visit our Web site frequently for updates on new and existing products. To receive some of this information directly, [Subscribe to Our E-mail Newsletter](#). We are already working on the next print edition of the *Handbook*, with an accompanying CD-ROM. We always welcome your comments and suggestions for improving the *Handbook* and our Web site, or for new products that you feel would be useful for your research.

Sincerely,

Richard P. Haugland

Richard P. Haugland, Ph.D.
President
Molecular Probes, Inc.

Top



Copyright © 1999 by Molecular Probes, Inc. [Legal Notices and Trademark Attributions](#).

CP

Chapter 20 — Indicators for Ca²⁺, Mg²⁺, Zn²⁺ and Other Metals

20.1 Introduction to Ca(2+) Measurements with Fluorescent Indicators

- Selection Criteria for Fluorescent Ca²⁺ Indicators
- [Products](#)

20.2 Fluorescent Ca(2+) Indicators Excited with UV Light

- Fura-2, Indo-1 and Derivatives
- Quin-2 and Derivatives
- Indicators with Intermediate Calcium-Binding Affinity
- Low-Affinity Calcium Indicators
- [Products](#)
- [Data Table](#)

20.3 Fluorescent Ca(2+) Indicators Excited with Visible Light

- Fluo-3, Rhod-2 and Related Derivatives
- Low-Affinity Calcium Indicators: Fluo-5N, Rhod-5N, X-Rhod-5N and Related Derivatives
- Calcium Green, Calcium Orange and Calcium Crimson Indicators
- Oregon Green 488 BAPTA Indicators
- Fura Red Indicator
- Calcein
- [Products](#)
- [Data Table](#)

20.4 Fluorescent Ca(2+) Indicator Conjugates

- Dextran Conjugates
- Lipophilic Derivatives for Detecting Near-Membrane Calcium
- [Products](#)
- [Data Table](#)

20.5 Aequorin: A Bioluminescent Ca(2+) Indicator

- Recombinant Aequorin
- Aequorin Expression Vectors
- Coelenterazine and Its Synthetic Analogs
- [Products](#)
- [Data Table](#)

20.6 Fluorescent Mg(2+) Indicators

- Magnesium Indicators Excited by UV Light
Magnesium Indicators Excited by Visible Light
- Products
Data Table

20.7 Fluorescent Indicators for Zn(2+) and Other Metals

- Applications of Ca²⁺ and Mg²⁺ Indicators for Detection of Zn²⁺ and Other Metals
- Indicators for Zinc
- Indicators for Copper
- Indicators for Iron
- Indicators for Mercury, Lead and Cadmium
- Indicators for Nickel and Cobalt
- Indicators for Aluminum and Gallium
- Indicators for Lanthanides
- Products
Data Table

20.8 Chelators, Calibration Buffers and Cell-Loading Reagents

- Caged Calcium and Caged Calcium Chelators
- Nonfluorescent Chelators
- Calcium Calibration Buffer Kits
- Influx Pinocytic Cell-Loading Reagent
- Ionophores
- Pluronic F-127: A Useful Dispersing Reagent
- Reagents for Investigating Calcium Modulation and Second Messenger Activity
- Products
Data Table

List of Tables

Table 20.1 — Summary of fluorescent Ca(2+) indicators

Table 20.2 — Coelenterazines and their properties

Table 20.3 — Ca(2+) and Mg(2+) indicator responses

Table 20.4 — Indicator responses to 25 microM solutions of metal ions

Table 20.5 — Ca(2+) affinities of BAPTA

Table 20.6 — Cell loading with fluo-3 and fluo-4 AM esters

Table 20.7 — Parallel performance comparison of fluo-3 and fluo-4 on Molecular Devices' FLIPR system

Table 20.8 — Comparison of *in vitro* and *in situ* K(d) values for fluo-3, fura-2 and indo-1

Table 20.9 — Response of fura-2 and indo-1 to some divalent cations other than Ca(2+) and Mg(2+)

List of Figures

Figure 20.1 — Fluorescence excitation spectra of fura-2 and bis-fura-2

Figure 20.2 — Fluorescence emission spectra of indo-1

Figure 20.3 — Fluorescence excitation spectra of BTC

Figure 20.4 — Ca(2+)-dependent fluorescence emission spectra of fluo-3

Figure 20.5 — Fluorescence emission spectra of a mixture of fluo-3 and Fura Red indicators

Figure 20.6 — Ca(2+)-dependent fluorescence emission spectra of Calcium Green-1, Calcium Green-2, Calcium Orange and Calcium Crimson indicators

Figure 20.7 — Ca(2+)-dependent fluorescence emission spectra of Calcium Green-5N and fluo-5N

Figure 20.8 — Ca(2+)-dependent fluorescence emission spectra of Oregon Green 488 BAPTA-1 and Oregon Green 488 BAPTA-2 indicators

Figure 20.9 — Propagation of a fertilization-induced Ca(2+) wave in a starfish egg visualized using microinjected Calcium Green-1 dextran

Figure 20.10 — Ca(2+)-dependent generation of luminescence by the aequorin complex

Figure 20.11 — Fluorescence excitation and emission spectra of mag-fura-2

Figure 20.12 — Fluorescence excitation and emission spectra of mag-indo-1

Figure 20.13 — Mg(2+)-dependent fluorescence emission spectra of Magnesium Green

Figure 20.14 — Fluorescence excitation spectra of APTRA-BTC

Figure 20.15 — Fluorescence emission spectra of a mixture of BTC-5N and TCPP

Figure 20.16 — Absorption spectra of BAPTA

Figure 20.17 — Fluorescence excitation spectra of benzothiazaz-1

Figure 20.18 — Principle of the Influx reagent cell-loading method

Figure 20.19 — CRE BAG 2 cells loaded with Alexa Fluor 488 hydrazide either by pinocytotic uptake in growth medium or in Influx hypertonic loading medium

Figure 20.20 — Fluorescence emission spectra at equal concentrations of fluo-3 and fluo-4

Figure 20.21 — Fluorescence emission spectra of mag-fluo-4

Figure 20.22 — Fluorescence emission spectra of X-rhod-1 and rhod-2

Figure 20.23 — Comparison of fluorescence intensity responses to Ca(2+) for fluo-3 and Calcium Green-1 indicators



Copyright © 1999 by Molecular Probes, Inc. Legal Notices and Trademark Attributions.

20.1 Introduction to Ca²⁺ Measurements with Fluorescent Indicators

Fluorescent probes that show a spectral response upon binding Ca²⁺ have enabled researchers to investigate changes in intracellular free Ca²⁺ concentrations using fluorescence microscopy, flow cytometry and fluorescence spectroscopy. ^{REF} These fluorescent indicators, most of which are derivatives of the Ca²⁺ chelators EGTA, APTRA and BAPTA, ^{REF} have evolved largely through the efforts of Roger Tsien and his colleagues and, more recently, through those of scientists at Molecular Probes.

Selection Criteria for Fluorescent Ca²⁺ Indicators

Molecular Probes offers the widest available selection of fluorescent Ca²⁺ indicators for detecting changes in intracellular Ca²⁺ over the range of <50 nM to >50 μ M (Table 20.1). Not only are we the primary supplier of fura-2, indo-1, fluo-3 and rhod-2, but we exclusively offer a number of other indicators for intracellular Ca²⁺. Our new fura-4F, fura-5F and fura-6F indicators provide increased response sensitivity to intracellular Ca²⁺ concentration in the 0.5–5 μ M range compared to fura-2. Our fluo-4, fluo-5F, fluo-5N, Oregon Green 488 BAPTA, Calcium Green, X-rhod-1 and Fura Red indicators allow Ca²⁺ detection over a wide concentration range and offer increased brightness and reduced phototoxicity. We also offer indicators that are conjugated to high- or low-molecular weight dextrans for improved cellular retention and less compartmentalization, as well as lipophilic Ca²⁺ indicators for possible use in studying near-membrane Ca²⁺ (Section 20.4). Molecular Probes strives to provide the highest-purity indicators available anywhere. The AM ester forms of most of our indicators are typically at least 95% pure by HPLC analysis, although purity often exceeds 98%. Furthermore, the AM esters of many of the Ca²⁺ and Mg²⁺ indicators are available in special packaging for more convenient handling and for reduced risk of deterioration during storage.

A number of factors should be considered when selecting a fluorescent Ca²⁺ indicator, some of which are summarized in Table 20.1 and include the:

- **Indicator form** (salt, AM ester or dextran), which influences the cell-loading method and affects the indicator's intracellular distribution and retention. The salt and dextran forms are typically loaded by microinjection, electroporation, patch-pipette perfusion or by using our Influx pinocytic cell-loading reagent (I-14400, I-14402, Section 20.8). In contrast, the cell-permeant acetoxymethyl (AM) esters can be passively loaded into cells, where they are cleaved to cell-impermeant products by intracellular esterases. For a discussion of ratiometric methods and AM ester loading, see Loading and Calibration of Intracellular Ion Indicators.
- **Measurement mode**, which is dictated by whether qualitative or quantitative ion concentration data, is required. Ion indicators that exhibit spectral shifts upon ion binding can be used for

rationetric measurements of Ca^{2+} concentration, which are essentially independent of uneven dye loading, cell thickness, photobleaching and dye leakage. Excitation and emission wavelength preferences depend on the type of instrumentation being used, as well as on sample autofluorescence and on the presence of other fluorescent or photoactivatable probes in the experiment.

- **Dissociation constant (K_d)**, which must be compatible with the Ca^{2+} concentration range of interest. Indicators have a detectable response in the concentration range from approximately $0.1 \times K_d$ to $10 \times K_d$. For ratiometric indicators, the Ca^{2+} response range is also somewhat dependent on the measurement wavelengths used. ^{REF} The K_d of Ca^{2+} indicators is dependent on many factors, including pH, temperature, ionic strength, viscosity, protein binding and the presence of Mg^{2+} and other ions. Consequently, K_d values for intracellular indicators are usually significantly higher than corresponding values measured in cell-free solutions (Table 20.8).

Intracellular calibration of Ca^{2+} indicators may be achieved either by manipulating Ca^{2+} levels inside cells using an ionophore or by releasing the indicator into the surrounding medium of known Ca^{2+} concentration via detergent lysis of the cells. We offer several control compounds and buffers for measuring and manipulating intracellular and extracellular Ca^{2+} , which are discussed in Section 20.8. These include caged- Ca^{2+} reagents and caged chelators (NP-EGTA, DMNP-EDTA and diazo-2), as well as Calcium Calibration Buffer Kits, BAPTA-derived buffers, ion-selective chelating polymers (Calcium Sponge products) and the Ca^{2+} ionophores A-23187 and its nonfluorescent analog, 4-bromo A-23187. Our reagents for probing Ca^{2+} regulation and second messenger activity are described in more detail in Chapter 18.

Reference Guides for Using Fluorescent Ca^{2+} Indicators

In order to meet the needs of researchers new to this technology, Molecular Probes offers selected books that provide surveys of fluorescent ion indicators and techniques for using them.

- *Methods in Cell Biology, Volume 40: A Practical Guide to the Study of Calcium in Living Cells* (M-7890), edited by Nuccitelli, is an indispensable guide for all researchers using fluorescent ion indicators.
- *Calcium Signaling Protocols (Methods in Molecular Biology, Volume 114)* (C-14945), edited by Lambert, provides optimized protocols for routine fluorometric Ca^{2+} measurements, as well as for confocal microscopy, subcellular Ca^{2+} imaging, Ca^{2+} channel activity determinations and detection of Ca^{2+} release from intracellular stores.
- *Fluorescent and Luminescent Probes for Biological Activity: A Practical Guide to Technology for Quantitative Real-Time Analysis, Second Edition* (F-14944), edited by Mason, is a comprehensive survey of optical probe techniques, including fluorescent ion indicators.

Other reviews of these indicators include those by Silver, ^{REF} Scheenen *et al.*, ^{REF} and Kao. ^{REF} Several earlier reviews on ion indicators also contain useful technical information. ^{REF}



Copyright © 1999 by Molecular Probes, Inc. Legal Notices and Trademark Attributions.